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Treatment (Preclearing and preadsorbing) of anti SmGPCR IgG

To eliminate *E. coli* antibodies as well as non specific antibody fraction (of S. tag, His tag, ..etc) that presents in polyclonal Ab that was prepared by injecting a recombinant tagged protein that was expressed in *E. coli* (prokaryotic system), you need to treat that antibody portion first with *E. coli* lysate (untransformed cell lysate) and/ or the purified recombinant S.tag/His tag induced empty plasmid in the same kind of expressing host cells.

Example:

I prepared a polyclonal Ab against a portion of the 3rd intracellular loop of SmGPCR by prepaing a recombinant His/S. tag il3 in pET30a vector and express it in Rosetta *E.coli* cells and inject the purified recombinant His/S. tagged il3 in rabbit. Thus, the developed polyclonal Ab will recognize (1) il3 epitopes, (2) His, S. tags epitopes and (3) some Rosetta *E. coli* proteins.

To increase the specificity to detect as much as only il3 epitopes, Idid the following:

(1) I grew non-transformed Rosetta cells (Chloroamphinicol + LB) and sonicate the pellet and use that lysate in **preclearing** the polyclonal Ab to eliminate E.coli Abs.

(2) I induced transformed rosetta cells with empty pET30a (i.e. no il3 insert) with 0.7M IPTG and solubilized the inclusion bodies in 6M urea+ His.bind buffer and purified it in His trap column (Novagen). I used the eluted stuff to **preadsorb** the polyclonal rabbit anti il3-SmGPCR IgG to eliminate S. tag and His tag antibodies.

Protocol:

Prior to using in IB or IFA, mix the working dilution of Ab in 1% skim milk+ TBST with 1:100 of bacterial lysate (4μg/μl) and 1:2000-1:200 of recombinant S/His tag peptide. Mix in end-over-end rotor for 1-1.5hr at room temperature and spin for 18min at 16,000 xg at 4C. Use the supernatant as a pretreated (precleared/preadsorbed) Ab. It is good to use a non treated Ab for comparison.